

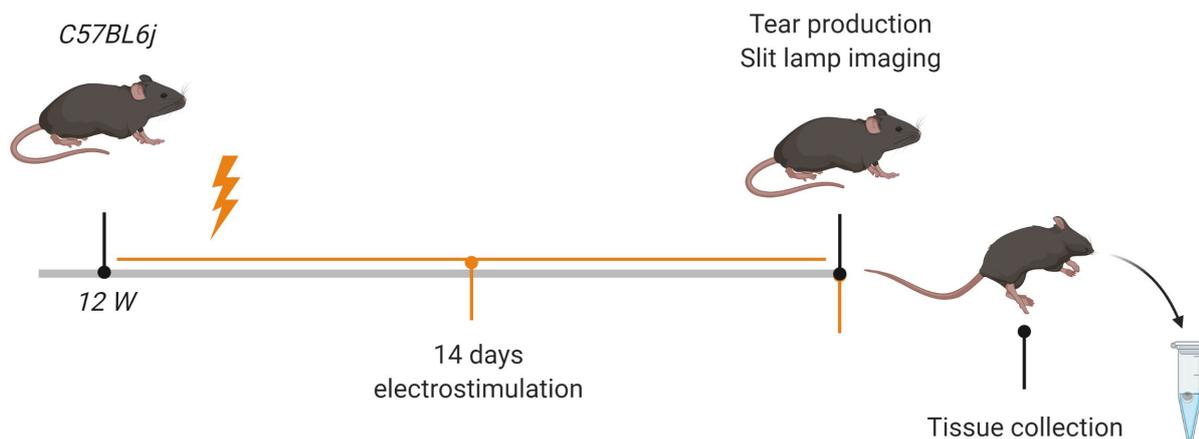
Transcorneal Electric Stimulation Therapy Induce Dry Eye by $[Ca^{2+}]_i$ Depletion of Goblet Cells and Reduce Mucin Production

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Background: Electrical stimulation (ES) is a therapeutic approach for numerous diseases including glaucoma and diseases of the retina. It delivers a low-intensity electric current to target tissue non-invasively through the application of electrodes to the cornea's surface (transcorneal ES [tES]) or the skin of the eyelid (transpalpebral ES [tpES]). In a recent clinical study, while experiencing an increase in visual acuity, 37% of the study subjects complained symptoms of dry eye after undergoing tES. Dry eye symptom is a multi-factoral condition that involves tearfilm stability, chronic ocular surface inflammation, corneal nerve structure and many more. Unfortunately, the clinical studies done on human subjects did not measure any objective parameters of dry eye, making it hard to distinguish the cause of the dryness sensation. The propose of this study is to understand the mechanism of dry eye symptoms induced by ES, and investigate the effect of electric on the ocular surface.



Method: 1) Female mice of C57BL6j, 12-week-old underwent tES in one eye for 14 days. At day 14, sodium fluorescence staining test was performed and observed under slit lamp for measurement of dry eye level. Phenol red thread test was used for tear production. 2) The animals were then be scarified and immunofluorescence staining of Mucin 4 (Muc4) was performed on fixed cornea tissue. 3) Primary human conjunctival goblet cells underwent ES for 1h at 300 μ A stimulated with carbacholine (10-4M) and change of intracellular $[Ca^{2+}]_i$ ($[Ca^{2+}]_i$) was measured.

Result: 14 days of tES, 4 min per day significantly increased the cornea florescence staining comparing to the adjacent eye. The sodium florescence staining was scored in superior, inferior, nasal, temporal and central regions of cornea, with no staining scored as 0, and a massive staining of florescence scored as 3. The scores of each region of the cornea are averaged to represent the score of dryness of the cornea. The score of sodium florescence stain in tES eye is 2.25 ± 0.19 while the adjacent eye scored 0.6 ± 0.24 ($p=0.001$), indicating that tES treatment could induce dry eye. The phenol red thread test for tear production showed no difference between tES eye and the adjacent eye ($p=0.40$), indicating that the dry eye induced by tES was not due to a lack of tear production. The immunofluorescence staining of the cornea whole mount showed a significant decreased signal of Muc 4, which indicated a decreased mucin production. 1h of ES in cultured goblet cells significantly down regulated the $[Ca^{2+}]_i$ increase induced by carbacholine ($p=0.03$).

Conclusion: tES 4 min per day for 14 days induced dry eye in female C57BL6j mice. The dry eye induced by tES is possibly a result of reduced mucin production from depleting $[Ca^{2+}]_i$ signals. Transpalpebral may be a better delivery of ES in future clinical application.